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Effects of intraperitoneal injection of Rofecoxib in a mouse model of ALS

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There is increasing evidence that inflammatory mechanisms are involved in the pathogenesis of amyotrophic lateral sclerosis (ALS). Inhibition of a key mediator of inflammation, cyclooxygenase 2 (COX-2), represents a promising therapeutic approach in ALS. Here we tested the *in vivo* effects of a specific COX-2 inhibitor, Rofecoxib, administered by intraperitoneal injection, in the $SOD1^{G93A\ G1H}$ mouse model of the familial form of ALS (fALS). Rofecoxib administration commenced at postnatal day 60 (P60), since the hallmarks of inflammation in the spinal cord were found to occur beyond this time-point in this mouse model of fALS. We found a significant but small delay in the onset of locomotor impairment in mice treated with Rofecoxib at the dose of 10 mg/kg of weight. However, survival was not effected by treatment. As prostaglandin E2 levels in spinal cord or in plasma were not reduced by Rofecoxib treatment, these results may suggest lack of sufficient bioavailability as the reason for the modest clinical changes observed.

Introduction

The role of inflammatory mechanisms in the pathogenesis of a variety of neurodegenerative conditions, not traditionally considered inflammatory in origin, such as Alzheimer's disease and Amyotrophic Lateral Sclerosis (ALS) is under intense investigation. It is generally believed that oxidative stress (Cleveland and Rothstein, 2001) and glutamate-mediated excitotoxicity (Shaw and Ince, 1997) are important mechanisms capable of causing degeneration of motoneurons in ALS. In fact Riluzole, the only medication approved for the treatment of human ALS, that extends survival by approximately 3 months is believed to act as a glutamate antagonist (Miller and Swash, 1996). In ALS, inflammation can contribute to both excitotoxicity and oxidative stress (McGeer and McGeer, 2002). Activity of a key inducible mediator of the inflammatory response, cyclooxygenase 2 (COX-2), can mediate pro-inflammatory cascades (Almer *et al.*, 2001) that in turn may induce motoneurons to die (Maihofner *et al.*, 2003). COX-2 activity leading to prostaglandin E2 (PGE_2) production can also induce release of glutamate from astrocytes (Sanzgiri *et al.*, 1999), thereby further contributing to excitotoxicity and motoneuron death (Urushitani *et al.*, 2001). This is particularly important as astrocytosis is a pathological feature of ALS (Dal Canto and Gurney, 1997). However, the contribution of astrocytes to the disease process may be a late event in disease progression (Hall *et al.*, 1998). This notion is supported by evidence that reactive astrocytes only become a prominent feature of the neuropathology in SOD1 transgenic mice beyond postnatal day 100 (P100), which corresponds to a stage of disease following the onset of symptoms (Hall *et al.*, 1998; Alexianu *et al.*, 2001). Significant levels of microglial activation however, have been found by Alexianu *et al.* (2001) at P80 in these transgenic mice. Hence microglia may play an earlier role in disease pathogenesis. This increase in reactive microglia may in fact be the underlying potentiator of PGE_2 levels (Minghetti and Levi, 1995; Slepko *et al.*, 1997). There are reports of increased CSF and serum levels of PGE_2 in sporadic ALS patients (Almer *et al.*, 2002; Ilzecka, 2003). Furthermore, increased COX-2 protein expression has been demonstrated in the spinal cord of both sporadic ALS patients (Almer *et al.*, 2001; Maihofner *et al.*, 2003) and the $SOD1^{G93A\ G1H}$ mouse model of familial ALS (Almer *et al.*, 2001; Ilzecka, 2003).

Recently, moderate therapeutic success has been reported *in vivo* with two COX-2 inhibitors, Celecoxib and Nimesulide, in the $SOD1^{G93A\ G1H}$ mouse model of fALS (Drachman *et al.*, 2002; Pompl *et al.*, 2003). Drachman and Rothstein (2000) have also shown, in an organotypic model of ALS, that specific inhibition of COX-2 leads to significant rescue of motoneurons. Klivenyi *et al.* (2004) have also demonstrated an

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additive beneficial effect of creatine with either orally administered Celecoxib or Refecoxib in SOD1 transgenic mice. Here we investigated the clinical effects of intraperitoneal administration of Rofecoxib, a specific COX-2 inhibitor, on the clinical progression of disease in $SOD1^{G93A\ G1H}$ mice. Two different doses of Rofecoxib were used to assess a dose-response relationship. We have previously reported some variation in disease parameters in different cohorts of SOD1 transgenic mice (Azari *et al.*, 2003). Therefore, we first investigated the induction of inflammatory mechanisms in our transgenic mice colony. This was carried out by determining the levels of soluble tumour necrosis factor receptor 1 (sTNFR1) and the spatiotemporal pattern of microglial activation in the spinal cords of transgenic mice from a closely related cohort to the one used in the *in vivo* trial of Rofecoxib. This allowed us to commence the treatment regimen prior to the time of activation of microglia, and hence allow for proper evaluation of Rofecoxib. We found no significant activation of microglia at P60, whilst they were activated significantly and in a widespread fashion through the white matter and grey matter of the spinal cord at P90 and beyond. Prominent upregulation of sTNFR1 was not seen at P60 in transgenic mice, whilst it was detected at P120. We also found no significant effect of Rofecoxib on survival of transgenic mice. However, a mild but significant delay in onset of motor deficit was seen in high dose Rofecoxib treated mice. Spinal cord or serum PGE_2 levels were not significantly altered by Rofecoxib treatment. These data suggest problems with bioavailability of Rofecoxib under these experimental conditions. Alternatively, they may suggest a reliance on the activation of microglia and hence COX-1 activity in the disease progression in ALS.

Materials and methods

Transgenic mice expressing the G93A mutation in the human SOD1 gene, derived from the B6SJL-TgN(SOD1-G93A)1Gur line (The Jackson Laboratory, Bar Harbor, USA), were bred and maintained as hemizygotes by mating transgenic males with wild-type B6SJL females. These experiments were carried out with the approval of Monash University Animal Ethics and Experimentation Committee (permit number: BAM/A/2000/8).

Microglial sections

Three groups of female SOD1 transgenic and three groups of their wild-type female littermates ($n = 3$ each) were killed by lethal injection (200 mg/kg, i.p.) using Nembutal® (Pentobarbitone Sodium; Rhone Merieux, Pinkemba, OLD, Australia) at 60, 90 and 120 days of age. Mice were intracardially perfused with phosphate buffered saline (PBS) at pH 7.4, followed by 4% paraformaldehyde in 0.2 M phosphate buffer at pH 7.4. Lumbar enlargements were dissected out and post-fixed over-night in 4% paraformaldehyde and subsequently cryopreserved overnight in 30% sucrose. The lumbar enlargements were then embedded transversely in OCT compound (TissueTek®, Sakura Finetek Inc., CA, USA) and frozen in isopentane on dry-ice. They were then serially sectioned for 1 mm of the lumbar enlargement at 10 μm on a cryostat. Sections were mounted on Superfrost/plus slides (Menzel-Gläser, Braunschweig, Germany) and stored at −20°C.

Microglial immunohistochemistry

Sections were post-fixed for 30 min with 4% paraformaldehyde. To demonstrate microglial activity, lumbar spinal cord sections were stained with the anti-CD11b antibody (Immunotech, Marseille Cedex, France). Briefly, the sections were washed thoroughly (3 × 10 min) following post-fixation and then incubated with 10% foetal calf serum (FCS) in PBS pH 7.4 with 0.3% Triton-X100 (blocking buffer) at room temperature (RT) for 2 h. The sections were then incubated overnight at 4°C with the anti-CD11b antibody (Immunotech) at a 1:50 dilution in blocking buffer. Following thorough washes in PBS (3 × 10 min), the sections were then incubated with a goat anti-mouse immunoglobulin conjugated to rhodamine (Chemicon) at a 1:40 dilution in blocking buffer at RT for 2 h and then washed in PBS (3 × 10 min). Sections were then cover-slipped using fluorescent mounting medium (DAKO, CA, USA) and images captured using a U-TV1X camera (Olympus, Ballerup, Denmark). Scanned digital images were then imported and processed in Adobe Photoshop.

Microglial activation was also assessed using biotinylated *Lycopersicon esculentum* (L.e.) lectin (EY Laboratories, San Mateo, CA, USA). Sections were washed thoroughly (3 × 10 min) following post-fixation and then incubated with 3% H_2O_2 in methanol for 30 min. in order to quench endogenous peroxidase activity. After washes in PBS (3 × 10 min) sections were incubated in blocking buffer at RT for 2 h, washed in PBS (3 × 10 min) and incubated overnight at 4°C with L.e. lectin at a 1:60 dilution in blocking buffer. After washes with PBS (3 × 10 min) sections were incubated for 1 h with avidin-labelled peroxidase (ABC Elite Kit; Vector Laboratories, Inc., Burlingame, CA, USA) and then washed again with PBS (3 × 10 min). They were then incubated with 0.05% DAB and 0.025% H_2O_2 diluted in PBS for 10 min, counterstained with haematoxylin, rinsed in distilled water, dehydrated in

absolute alcohol, cleared in xylene and coverslipped with the DPX synthetic resin mounting medium. All staining involved the use of a negative control (secondary antibody alone) in order to evaluate the specificity of the antibodies.

Quantification of microglial activation

Numbers of microglia were manually counted in white and grey matter of the spinal cord at 400× final magnification. For each animal, eight cross sections were examined and microglia were counted only if they possessed a round purple nucleus (haematoxylin stain) that was surrounded by brown cytoplasmic processes (L.e. lectin). The area of the white matter and the grey matter for each cross section was measured using CAST version 2.1.1 (Olympus, Ballerup, Denmark). This allowed for the density of microglia (microglia/mm^2) in the white matter and grey matter to be calculated. For each animal the calculated densities of the eight cross sections were averaged. Subsequently, the groups mean densities for the white and grey matter were calculated. The data are expressed as mean ± SD. Differences between transgenic SOD1$^{G93A\ G1H}$ mice and wild-type mice at P60, 90 and 120 were statistically evaluated with a paired Student's *t*-test.

Western blot analysis

In an attempt to correlate microglial activity with the progression of disease in SOD1$^{G93A\ G1H}$ mice immunoblotting was performed using a sTNFR1 antibody. Briefly, freshly dissected, snap frozen, grinded and lysis-buffer-dissolved protein extracts (20 μg) of the lumbar enlargement, from P60 and P120 SOD1$^{G93A\ G1H}$ and wild type mice were solubilized in 20 μl of Tris-HCl (pH 6.8), 4% (w/v) SDS, 2% (w/v) glycine, 0.01% (w/v) bromophenol blue, 20% (v/v) glycerol and 10% (v/v) β-mercaptoethanol. Samples were heated at 95°C for 5 min and subsequently run through 12.5% polyacrylamide gels at 50 V for 30 min and then 90 V for 1 h. Proteins were then electroblotted onto Protran nitrocellulose membranes (Medos Company, Mount Waverley, Australia) – 0.2 μm pore diameter. Membranes were then blocked with 5% (w/v) defatted milk, 0.1% (v/v) Tween-20, and 0.02% (w/v) NaN_3 in TBS (pH 8.0) for 1 h and then incubated overnight at 4°C with a monoclonal antibody specific for sTNFR1 (Sigma, Castle Hill, Australia) diluted 1:50 in 5% skin milk at pH 7.4. Membranes were washed three times in TBS-Tween-20 (pH 8.0) for 10 min each, incubated with HRP-conjugated secondary antibodies (1:2500) diluted in TBS for 1 h, washed four times in TBS for 10 min each, and protein bands were visualized after reaction of the secondary antibody-conjugate with the chemiluminescence substrate in the Lumi-Light kit (Roche Diagnostics, Nunawading, Australia) and the subsequent exposure of High Performance chemiluminescence film to the membrane surface (Amersham pharmacia biotech, Buckinghamshire, England) in the dark.

i.p. Rofecoxib trial

Two groups of female SOD1$^{G93A\ G1H}$ mice ($n = 6$ each) and two groups of their female wild-type littermates ($n = 5$) were injected with either low or high doses of Rofecoxib dissolved in physiological saline containing 5% Triton X 100 (BioRad, CA, USA). The high dose of 10 mg/kg of Rofecoxib was used as this equates to the maximum human daily dose. A low dose of 2.5 mg/kg was also used for the purpose of comparison. Rofecoxib was dissolved in 5% Triton X 100 in saline. Another control group of female transgenic mice ($n = 5$) was injected with physiological saline containing 5% Triton X 100. Rofecoxib (Merck Sharp & Dohme, NJ, USA) or saline were administered thrice weekly by intraperitoneal injection. Treatment commenced at the age of 60 days (P60) and continued until the mice reached a stage where one or both hindlimbs became paralysed, at which point they were killed as outlined above.

Locomotor status of the mice was determined weekly using a Rotarod apparatus in which the mice were made to balance on a rod rotating at 16 r.p.m. for a maximum period of 180 s. Weight loss is normally observed in SOD1$^{G93A\ G1H}$ mice with disease progression. Therefore, the mice were weighed weekly to further monitor their clinical status. Following the lethal injections, the mice were perfused intracardially with PBS containing 5% Heparin. Blood samples were taken by cardiac puncture immediately prior to perfusion.

Lumbar spinal cord enlargements were dissected immediately following perfusion and snap-frozen in liquid nitrogen. PGE_2 extraction was carried out by a modification of the method of Narumiya *et al.* (1982). Briefly, 25–50 mg of spinal cord was combined with tritiated PGE_2 (1000 c.p.m.) in a 1.5 ml microcentrifuge tube and allowed to come to 4°C. The tissue was then crushed using plastic pastels designed to tightly fit the tube base. After creating a smooth tissue paste, five volumes of ethanol was added and the tissue was further dispersed in the ethanol using the pastel. Following centrifugation for 10 min the ethanol was removed and the pallet was extracted twice more using the same volume of ethanol for each extraction. The three supernatants were combined and the pallet retained for protein analysis. The ethanolic supernatants were dried under nitrogen at 37°C. PGE_2 in the dried extracts was resuspended in 250 μl of assay buffer. The mean

recovery of tritiated PGE_2 was 84.5 ± 2.2% and correction for the recovery of each sample was included in the final calculation of PGE_2 values Plasma was assayed without extraction. PGE_2 in spinal cord tissue extracts or plasma (100 μl) was subjected to methyloximation overnight by the method of McLaren *et al.* (1996). The stable methyloxime derivative of $PGE_2$was measured using a radioimmunoassay described previously (McLaren *et al.*, 1996). The intra-assay coefficient of variation for the assay was 5.6%.

One-way ANOVA with Tukey's *post hoc* test was carried out to analyse the Rotarod and PGE_2 data. Repeated measures ANOVA was used for weight data, and Kaplan–Meier analyses were performed for assessment of survival data. These statistical tests were carried out using Prism software (Version 3.0; GraphPad Software Inc., San Diego, CA, USA). All group values are expressed as mean ± SEM.

Results

Figure 1a demonstrates activated microglia in a P120 transgenic mouse using the CD11b immunostain as a marker of activated microglia. Fig. 1b depicts white matter and grey matter regions in the lumbar enlargement, sampled for microglial density counts. Figure 1(c and d), depict the up-regulated microglial activation at P60, P90, and P120 stages of disease progression in the transgenic $SOD1^{G93A\ G1H}$ mice. At the P60 time-point (representing the pre-symptomatic stage of disease) no statistically significant difference in the density of activated microglia was observed for either the white or the grey matter. In fact, minimal microglial activity was observed in either transgenic or wild-type groups. However, at P90 and P120, (representing disease onset and end-stage disease respectively) we found a substantial increase in the number of activated microglia in transgenic mice. This correlated with a strong upregulation of sTNFR1 at P120 (Fig. 1e) as a marker of inflammation. The white matter of P90 and P120 $SOD1^{G93A\ G1H}$ mice displayed a respective fivefold and 13-fold increase in microglial density when compared with that of $SOD1^{G93A\ G1H}$ P60 mice. This trend was similar for the grey matter as well. At P90, $SOD1^{G93A\ G1H}$ mice displayed a sevenfold increase and at P120 the increase was 16-fold. Interestingly, activated microglia were seen almost evenly distributed throughout the cross section of the spinal cord spanning the white and the grey matter. These data demonstrate that endogenous inflammatory mechanisms are initiated at a time beyond P60. Based on these results we commenced the Rofecoxib trial at 60 days of age.

Wild-type mice in the trial consistently gained weight through the period of the experiment, whereas the $SOD1^{G93A\ G1H}$ transgenic mice, as expected, began to lose weight after 102 days of age (Fig. 2a). The difference between the wild-type groups and the transgenic groups was statistically significant ($P < 0.01$) whereas that between the transgenic mice treated with either Rofecoxib or saline was not.

Figure 2b demonstrates a clear delay in the onset of motor deficit as detected by the rotarod test in the high dose Rofecoxib-treated transgenic mice. In this group of mice, the onset of motor deficit was postnatal day 102. At this time Rotarod performance of this group was 180 ± 0 s. Whereas the performance of low dose Rofecoxib treated and saline treated transgenic mice at the same time-point were 100.1 ± 29.3 and 154 ± 25.86 s respectively. This moderate delay in the onset of performance deficit was statistically significant ($P < 0.05$). Nevertheless, Kaplan–Meier analysis of survival data in Fig. 2c shows that treatment with either dose of Rofecoxib failed to exert a significant beneficial effect on survival of the transgenic mice.

Surprisingly, as can be seen in Fig. 3, PGE_2 levels in spinal cord or in serum were not altered by Rofecoxib treatment. Spinal cord PGE_2 levels (Fig. 3a) were: 175.4 ± 25.72 (low dose transgenic), 162 ± 13.85 (high dose transgenic) 116.4 ± 12.11 (low dose wild-type) 115.5 ± 27.8 (high dose wildtype), and 122.5 ± 38.76 (saline treated transgenic mice). The corresponding plasma values (Fig. 3b) were: 2.73 ± 1.25 (low dose transgenic), 5 ± 1.59 (high dose transgenic), 1.51 ± 0.72 (low dose wildtype), 1.14 ± 0.38 (high dose wildtype), and 2.66 (single saline treated transgenic). There was no significant difference in PGE_2 levels in the spinal cord ($P = 0.25$), or serum ($P = 0.17$) between transgenic and wild-type mice treated with Rofecoxib or saline.

Discussion

It is now well documented that reactive glia play a significant role in the onset and progression of the classical hallmarks of disease in ALS. For instance, it has been shown by high-density oligonucleotide arrays that glial inflammatory genes are induced at the onset of clinical symptoms, postnatal day 90, in $SOD1^{G93A}$ mice (Olsen *et al.*, 2001). In the present study, we have shown that microglia are uniformly activated in the lumbar enlargement of the spinal cord at the onset of clinical symptoms in SOD1 transgenic mice and before significant motoneuron dropout. It has been suggested that during the final stages of ALS, microglia secrete neurotoxins that accelerate the process of neural degeneration (Hall *et al.*, 1998). This hypothesis is consistent with localization of microglia in the vicinity of motoneurons in the anterior horn of the spinal cord

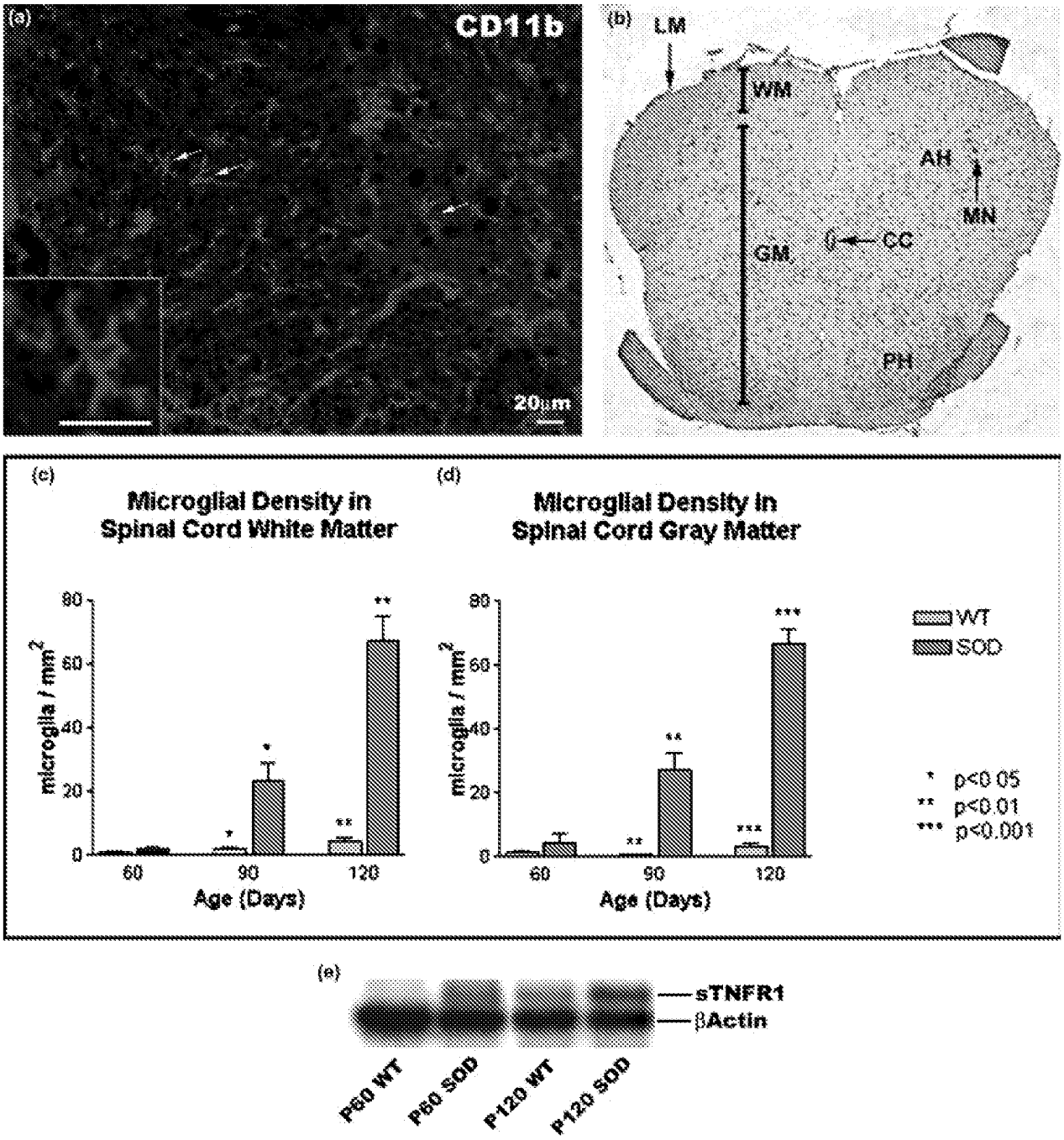

Figure 1 Measure of cellular and molecular markers of inflammation in the transgenic p120 $SOD1^{G93A}$ spinal cord (a) CD 11b stain for microglia in a P120 transgenic mouse. (b) Photomicrograph of a typical H &E stained cross section of the spinal cord lumbar enlargement to demonstrate areas used for microglial counts. WM, white matter; GM, grey matter; LM, Leptomeninges; AH, anterior horn; PH, posterior horn; CC, central canal; and MN, motor neuron. (c and d) Comparison of the densities of activated microglia at p60 ($n = 3$), p90 ($n = 3$) and p120 ($n = 3$) in transgenic and wild-type mice. Data given as mean ± SEM. The asterisks indicate statistical significance derived from the Student's *t*-test. (e) Protein levels of sTNFR1 in spinal cords of transgenic and wild-type mice at P60 and P120 time-points. Prominent upregulation is shown to occur at P120 toward disease end-stage.

(Hall *et al.*, 1998). However, it does not account for the significant accumulation of these cells in the white matter. Perhaps, microglial accumulation in the white matter is an indication that these cells are mopping up axonal debris from degenerating axons of primary motoneurons descending down their efferent tracts (Cluskey and Ramsden, 2001; Raineteau *et al.*, 2001). This is consistent with the neuropathology observed in

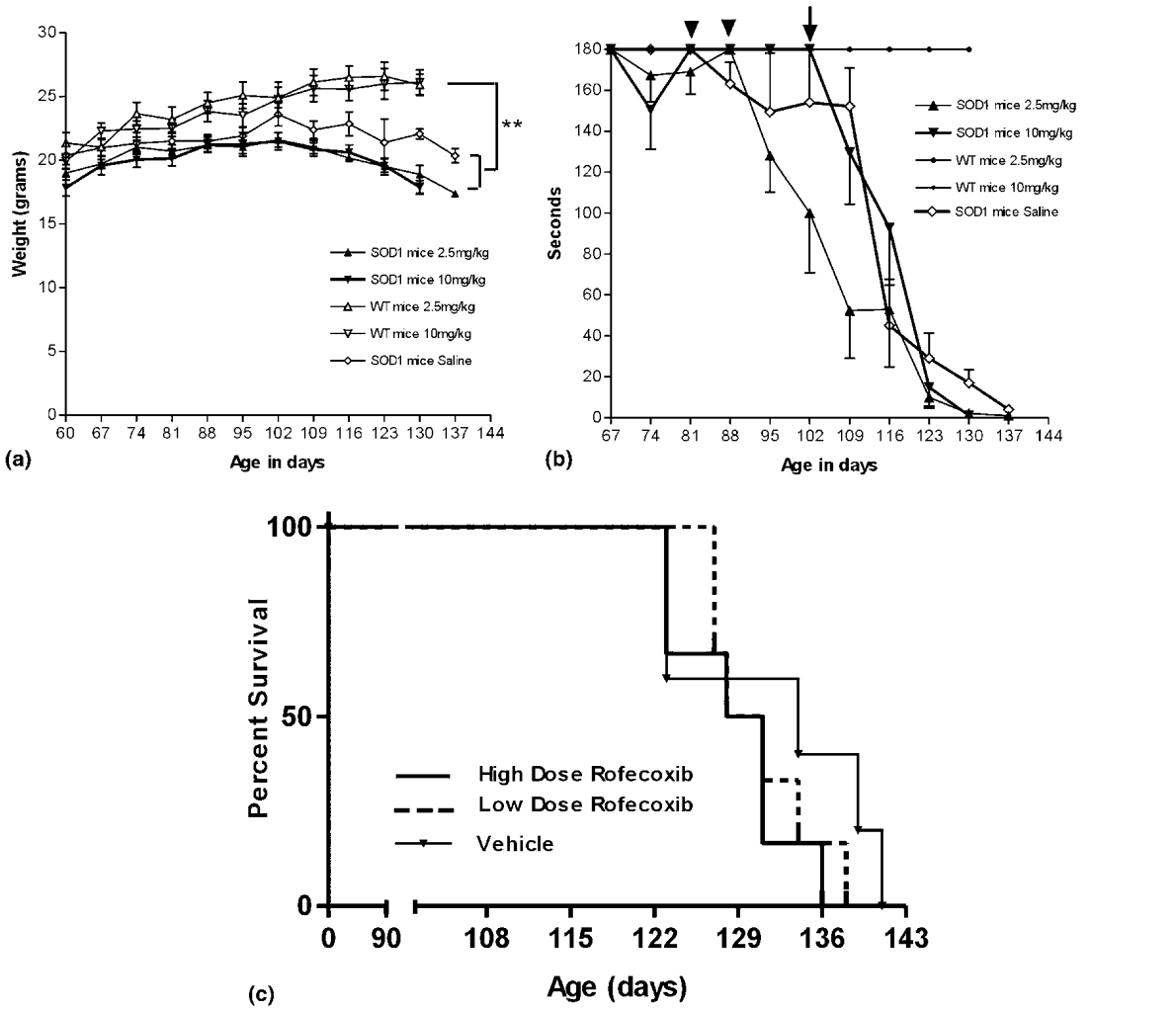

Figure 2 (a) Weekly weight measurements of the mice (mean ± SEM) showed a significant expected difference between wild-type and SOD1 transgenic mice ($P < 0.01$) as they aged. (b) There is a small but significant delay ($P < 0.05$) in the onset of motor deficit (mean ± SEM) in the high dose Rofecoxib group (arrow) compared with other transgenic groups (arrowheads) as determined by the Rotarod test. Panel (c) demonstrates that there is no significant prolongation of survival as a result of treatment with Rofecoxib at either dose used.

the $SOD1^{G93A}$ transgenic mouse model of ALS, which involves axonal loss and atrophy of the spinal cord white matter (Dal Canto and Gurney, 1995). Alternatively, microglia may be activated non-specifically in a more global manner without requirement of association with degenerating motoneurons.

One of the most significant inducers of cellular and molecular responses in the inflamed spinal cord is the upregulation of the cyclooxygenase (COX) enzyme. There are two distinct COX isoenzymes, COX-1 and COX-2 both of which can up-regulate conversion of arachidonic acid to prostaglandins. COX-2 is the inducible isoform, which is responsive to inflammatory stimuli such as cytokines. COX-2 levels have been shown to be elevated in the spinal cords of both the $SOD1^{G93A}$ mice and sporadic human ALS patients (Almer *et al.*, 2001).

Therefore, inhibition of the inflammatory cascade in general and COX-2 activity in particular represents a promising approach for the treatment of ALS. Inhibition of COX-2, may contribute to motoneuron survival in ALS by suppression of pro-inflammatory cascades. In addition to suppression of COX enzyme activity, there is *in vitro* evidence that non-steroidal anti-inflammatory drugs (NSAIDs) suppress mitogen-induced activation of the transcription factor NFκB, which is the master gene regulator of inflammatory mediators including COX (Paik *et al.*, 2000). Therefore, administration of Rofecoxib, an NSAID, can down-regulate the pro-inflammatory response through

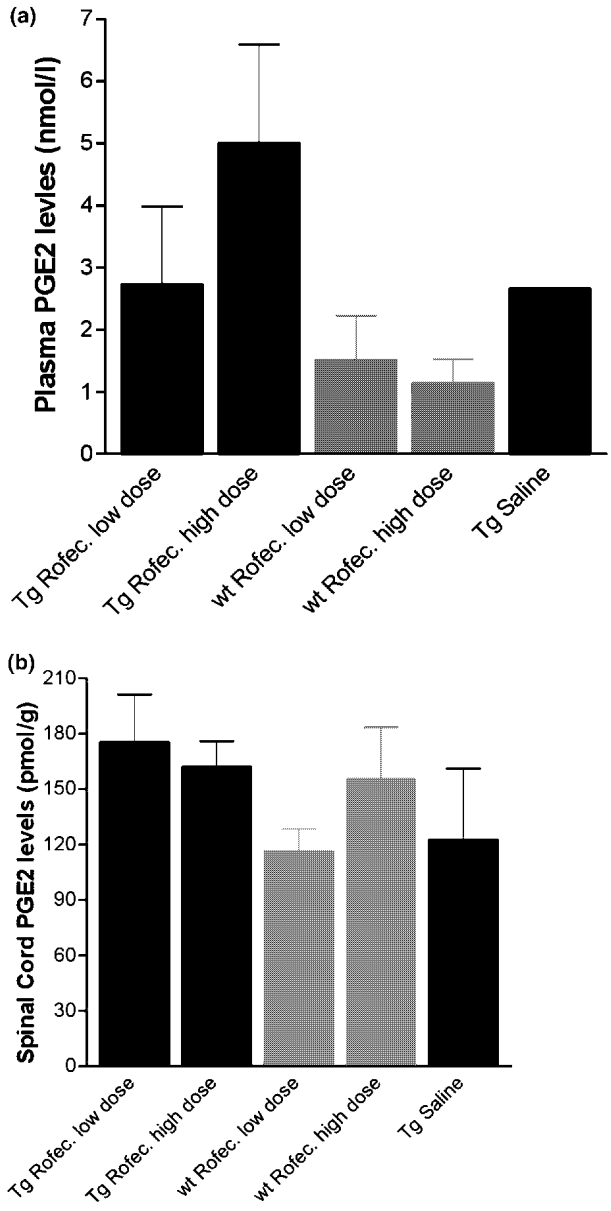

Figure 3 Plasma (a) and spinal cord (b) levels of PGE2 showed no modulation of COX-2 activity as a result of treatment with the COX-2 inhibitor Rofecoxib.

modulation of NFκB activity, inhibiting COX-2, and thereby down-regulating prostaglandin synthesis. This decrease in COX-2 activity can further abrogate the production of the proinflammatory cytokines thereby inhibiting microglial activation. Indeed, Drachman *et al.* (2002) have shown a significant reduction in the number of activated microglia in the spinal cords of $SOD1^{G93A\ G1H}$ mice treated with another NSAID, Celecoxib. An alternate mechanism by which COX-2 inhibitors can lead to motoneuron survival in ALS is by reduction of glutamate release by astrocytes (Sanzgiri *et al.*, 1999), thereby limiting glutamate excitotoxicity.

However, under the experimental conditions deployed in this study, Rofecoxib, failed to exert a significant beneficial effect on survival in $SOD1^{G93A\ G1H}$ mice. Injected Rofecoxib at the thrice-weekly dose of 10 mg/kg only caused a small but significant delay in the onset of motor deficit as detected by the Rotarod test. These results are in contrast to recent reports that show relatively greater beneficial effects following intake of two other anti-inflammatory agents (Celecoxib and Nimesulide) supplied *ad libitum* in the feed (Drachman *et al.*, 2002; Pompl *et al.*, 2003). It is possible that oral administration through the feed allows either better absorption or more sustained plasma levels of the therapeutic substance, hence leading to more beneficial clinical effects. Recently, Klivenyi *et al.* (2004) have shown that oral administration of Rofecoxib has beneficial effects in $SOD1^{G93A}$ mice. However it should be noted that the dose used in their study was threefold higher than the highest dose used in the present study. As the highest dose used in our study corresponds to the human dose used in clinical medicine (Drachman *et al.*, 2002), it is doubtful that higher doses would be safe in humans. This is particularly true given the fact that 'specific COX-2 inhibitors' such as Rofecoxib, have been shown to also inhibit COX-1 and thereby cause side effects such as gastrointestinal toxicity (Tacconelli *et al.*, 2004).

Kunz and Oliw (2001) also administered Rofecoxib by the intraperitoneal route and demonstrated its anti-inflammatory effect in the rat hippocampus after Kainic acid administration. However, these investigators used dimethylsulfoxide (DMSO) (10 mg/ml) as the vehicle rather than 5% Triton X 100 used in this study. It should be noted that Triton X 100 and similar solvents have been used as vehicles for *in vivo* drug delivery (Strauss *et al.*, 1978; Turner *et al.*, 2003). It is possible that a better solubility in DMSO may have led to more effective absorption of Rofecoxib. As we found no reduction of PGE_2 levels in the plasma or spinal cord, it is probably that under these experimental conditions, Rofecoxib does not reach sufficient levels in the CNS and therefore fails to exert a significant anti-inflammatory effect that could have prolonged survival of these mice. The results of our study highlight the importance of on-going research for the effective modulation of inflammatory mechanisms in ALS with particular emphasis on optimization of drug solubility and dose, the route of administration, and drug choice.

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